

# Cytological and histopathological abnormalities of the cervix in genital *Chlamydia trachomatis* infections

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**SUMMARY** Since genital infection with *Chlamydia trachomatis* may be associated with cervical abnormalities 160 patients with glandular ectopia attending a gynaecological outpatient clinic were examined for antibodies against *C trachomatis*, the presence of *C trachomatis* infection, and cytological and histopathological abnormalities of the cervix.

A significantly higher incidence of histological dysplasia was found in women with glandular ectopia who had antichlamydial antibodies than in those without.

## Introduction

*Chlamydia trachomatis* is a well established cause of non-gonococcal urethritis in men<sup>1-3</sup> and is often isolated in the sexual partners of men with chlamydial urethritis<sup>4</sup>; however, little is known about the natural history of chlamydial infection in women. Recent reports have indicated a possible association between *C trachomatis* infection and cervical abnormalities.<sup>5-8</sup>

In a previous study<sup>9</sup> of 106 women who were admitted to the department of obstetrics and gynaecology, University of Bologna, for reasons other than genital tract infections, 22 (20.7%) had serum antibodies against *C trachomatis* detected by a single-antigen immunofluorescence (IF) test. These women also had a higher incidence of glandular ectopia (63.7%) than those without antichlamydial antibodies (34.5%). These results prompted us to investigate women with glandular ectopia for genital chlamydial infection, for antichlamydial antibodies, and for histopathological abnormalities of the cervix.

## Patients and methods

### STUDY POPULATION

One hundred and sixty women (aged between 19 and 42 years) with glandular ectopia of the cervix

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detected by colposcopy were studied. At the first clinic attendance the following data were recorded: age and marital state, history of sexually transmitted diseases (STD) within the last year, parity and current contraceptive methods, and the number of sexual contacts within the last six months.

### COLLECTION OF SPECIMENS

Specimens were collected from the vagina for microscopy and culture for *Trichomonas vaginalis* and *Candida albicans* and from the cervix for microscopy and culture for *Neisseria gonorrhoeae* and for cell culture for *C trachomatis*. Blood samples were taken for serological tests for *C trachomatis*.

### IDENTIFICATION

Gram-stained cervical specimens were examined for intracellular diplococci and cultured for *N gonorrhoeae* on Thayer-Martin medium. Vaginal specimens for *T vaginalis* were directly examined at  $\times 400$  magnification. Cultures for *C albicans* were performed on Sabouraud's medium.

### CELL CULTURE FOR *C TRACHOMATIS*

The isolation of *C trachomatis* in cell culture has been described in detail elsewhere.<sup>10</sup> Specimens were collected with sterile alginate swabs in 2 ml of collecting medium (Eagle's minimum essential medium plus glutamine, inactivated fetal calf serum 10%, glucose 0.27 mg/ml, fungizone 2  $\mu$ g/ml, gentamicin 20  $\mu$ g/ml, and vancomycin 10  $\mu$ g/ml). The suspension was inoculated into IUDR-pretreated

McCoy cells<sup>11</sup> and centrifuged at 3000 rev/min for one hour.

#### SEROLOGY

The presence of antichlamydial antibodies was determined by an indirect immunofluorescence test using a modification of the technique of Richmond and Caul.<sup>12-13</sup> McCoy cells grown on Lab-Tek chamber slides were infected with *C trachomatis* L-2 with an inoculum predetermined to give about 30 inclusions per chamber. After 48 hours the cells were fixed with acetone. Serial two-fold dilutions of each serum sample were put on the cells and incubated for 45 minutes at 37°C. The slides were then washed three times and dried. Fluorescein-conjugated anti-human immunoglobulin G was added and the slides incubated for 45 minutes at 37°C, washed, counter-stained with Evans blue 0.01% in distilled water, mounted in glycerin, and observed in a Leitz UV microscope at  $\times 400$  magnification.

#### CERVICAL CYTOLOGY

Cytological smears were obtained from cervical scrapings, stained by the Isaac-Wurch technique,<sup>14</sup> and classified according to Papanicolaou<sup>15</sup>: class I, negative; class II, atypical (IIa mild, IIb moderate, IIc severe atypia); class III, suspicious; and class IV and V, positive.

#### HISTOLOGY

Biopsy specimens of the cervix were taken under colposcopic vision, fixed in formalin, and stained with haematoxylin and eosin.

#### COLPOSCOPY

The colposcopic findings at a magnification of  $\times 12-20$  were classified according to the First World Congress of Colposcopy (1972).<sup>16</sup>

#### STATISTICAL ANALYSIS

Statistical significance was evaluated by  $\chi^2$  tests with Yates's correction.

#### Results

One hundred and fifteen (71.8%) of the 160 women studied were married; 45 (28.1%) were nulliparous. Two patients had been admitted to the study as sexual contacts of men with non-gonococcal urethritis. The mean age at the first coitus was 19 years. During the six months before examination 140 (87.5%) women had had one sexual partner only, and 20 (12.5%) had had two or more.

#### MICROBIOLOGY

*C trachomatis* was recovered from nine (5.6%), *C albicans* from 32 (20%), *T vaginalis* from nine

(5.6%), and *N gonorrhoeae* from one (0.6%) of the 160 patients. None of these pathogens could be recovered from 109 (68.1%) patients. Six (3.7%) patients had a history of STD within the previous year.

#### SEROLOGY

Antichlamydial antibodies were detected at a titre  $\geq 1/8$  in 101 (63.1%) patients (table I), whereas 59 (36.8%) patients had no detectable antibodies.

TABLE I Antichlamydial antibody titres detected by immunofluorescence (IF) in 101 patients with glandular ectopia

IF titres	No	%
$\leq 1/8$	38	37.6
1/16	28	27.7
1/32	17	16.8
1/64	12	11.8
1/128	6	5.9

#### COLPOSCOPY

Simple glandular ectopia was present in 62 (38.7%) women and an area of ectopy with irregular epidermisation in 24 (15%).

#### CYTOLOGY

Papanicolaou cervical smears were classified as negative (class I) in 96 (60%), inflammatory (class II) in 42 (26.2%), inflammatory with moderate cellular atypia (class IIb) in 20 (12.5%), and suspicious (class III) in two (1.2%) patients (table II). Compared with seronegative women, patients with antichlamydial antibodies (titre  $\geq 1/8$ ) had a higher incidence of cervical abnormalities (table II). Of 24 cases with irregular epidermisation 20 (83.3%) had antichlamydial antibodies, and of 22 women with cellular atypia (class IIb and class III) 18 (81.8%) had antichlamydial antibodies. Moreover, among the seropositive patients only those with an antibody titre  $\geq 1/16$  showed an area of ectopy with irregular epidermisation and moderate (class IIb) or suspicious cellular atypia (class III).

After these results had been obtained, the 63 patients with antichlamydial antibodies at a titre  $\geq 1/16$  and the 59 seronegative patients were asked to attend again for further investigation. Only 55 of the 63 seropositive (titre  $\geq 1/16$ ) and 28 of the 59 seronegative women did so. The study was carried out in the same way as before; in addition a direct biopsy of the cervix was performed under colposcopic vision.

#### HISTOLOGY

The histological findings in the two groups of women are given in table III. The distribution of other

TABLE II Colposcopic and cytological findings in patients with antichlamydial antibodies (titre  $\geq 1/8$ ) and in those without

	Antichlamydial antibodies:					
	Present		Absent		Total	
	No	%	No	%	No	%
<i>Cervical abnormalities</i>						
Colposcopy						
Simple ectopy	48	47.5	14	23.7	62	38.7
Ectopy with polyps	7	6.9	1	1.7	8	5
Ectopy with regular epidermisation	26	25.7	40	67.8	66	41.2
Ectopy with irregular epidermisation	20	19.8	4	6.8	24	15
Total	101		59		160	
Cytology						
Class I (negative)	57	56.4	39	66.1	96	60
Class II (inflammatory)	26	25.7	16	27.1	42	26
Class IIb (moderate atypia)	16	15.8	4	6.8	20	12.5
Class III (suspicious)	2	1.9			2	1.2
Total	101		59		160	

possible causes of cervical dysplasia among the seropositive and seronegative women is shown in tables IV and V.

### Discussion

In this study of women with glandular ectopia antibodies against *C trachomatis* were present in 101 (63.1%) patients at a titre  $\geq 1/8$  and in 63 (39.4%) patients at a titre  $\geq 1/16$ . Cervical abnormalities were present only in patients with antibodies at a titre  $\geq 1/16$ ; 20 patients showed glandular ectopia with irregular epidermisation and 18 cellular atypia (class IIb and III). In the women without antichlamydial antibodies the incidence of these abnormalities was significantly lower than in the women with antichlamydial antibodies ( $P < 0.005$ ). These findings were confirmed by histological examination; 19 of 55 seropositive patients who had a biopsy of the cervix performed showed cervical dysplasia whereas only one of 28 seronegative women had mild cervical dysplasia. This difference was significant ( $P < 0.005$ ). The difference in willingness to undergo cervical

biopsy among the seropositive and seronegative patients (55 of 63 and 28 of 59 respectively) may be because the latter patients came from rural areas and the former mostly from urban areas, which were nearer to the hospital.

Cervical dysplasia has long been frequently associated with parity and multiple sexual partners; similarly, vaginitis due to *T vaginalis* and *C albicans* is often associated with mild dysplasia. In the patients studied these possible causes of mild dysplasia were found in both seronegative and seropositive patients without significant differences between these groups.

Although these results do not prove that *C trachomatis* is an aetiological factor in the development of cervical dysplasia, because *C*

TABLE IV Relationship of clinical data from 160 patients studied to the presence of antichlamydial antibodies

	Antichlamydial antibodies:			
	Present		Absent	
	No	%	No	%
<i>Clinical data</i>				
Methods of contraception				
None	40	39.6	21	35.6
Oral contraceptive	28	27.7	17	28.8
IUCD	18	17.8	11	18.6
Diaphragm	9	8.9	6	10.2
Others	6	5.9	4	6.8
Marital state				
Married	75	74.2	40	67.8
Unmarried	26	25.7	19	32.2
Obstetric history				
No pregnancy	32	31.6	25	42.4
Pregnancy	69	68.3	34	57.6
No of sexual partners*				
One	90	89.1	50	84.7
Two or more	11	10.9	9	15.2
History of previous STD†	4	4.0	2	3.4

\* During the last six months

† During the last year

TABLE III Histological results of patients with antichlamydial antibodies (titre  $\geq 1/16$ ) and of those without

	Antichlamydial antibodies:			
	Absent		Present	
	No	%	No	%
<i>Histology</i>				
Normal mucosa	1	3.6		
Chronic cervicitis	26	92.9		
Glandular cystic cervicitis			26	47.3
Chronic ulcerated cervicitis			3	5.4
Polyps of the cervix			7	12.7
Mild dysplasia	1	3.6	15	27.3
Moderate dysplasia			2	3.6
Severe dysplasia			2	3.6
Total	28		55	

TABLE V Distribution of micro-organisms isolated from patients with antichlamydial antibodies and from those without

Micro-organisms	Antichlamydial antibodies:			
	Present		Absent	
	No	%	No	%
<i>C. trachomatis</i>	9	8.9		
<i>N. gonorrhoeae</i>	1	1.0		
<i>C. albicans</i>	18	17.8	14	23.7
<i>T. vaginalis</i>	6	5.9	3	5.1
No pathogens	67	66.3	42	71.2
Total	101		59	

*trachomatis* was isolated from the cervix of only nine patients, they do show a significantly higher incidence of cervical dysplasia in seropositive than in seronegative women, thus confirming that some link may exist between the two phenomena.

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#### References

1. Bowie WR, Wang S, Alexander ER, Holmes KK. Etiology of non-gonococcal urethritis. In: Hobson D, Holmes KK, eds. *Non-gonococcal Urethritis and Related Infections*. Washington DC: American Society for Microbiology, 1977; 19-29.
2. Holmes KK, Handsfield HH, Wang SP, Wentworth BB, Turk M, Anderson JB, Alexander ER. Etiology of non-gonococcal urethritis. *N Engl J Med* 1975; **292**: 1199-205.
3. Schachter J. Chlamydial infections. *N Engl J Med* 1978; **298**: 490-5.
4. Woolfitt JM, Watt L. Chlamydial infection of the urogenital tract in promiscuous and non-promiscuous women. *Br J Vener Dis* 1977; **53**: 93-5.
5. Schachter J, Hill EC, King EB, Coleman VR, Jones P, Meyer KF. Chlamydial infection in women with cervical dysplasia. *Am J Obstet Gynecol* 1975; **3**: 753-7.
6. Paavonen J, Vesterinen E, Meyer B, Saikku P, Suni J, Puroila E, Saksela E. Genital *Chlamydia trachomatis* infection in patients with cervical atypia. *Obstet Gynecol* 1979; **54**: 289-91.
7. Carr M, Hanna L, Jawetz E. Chlamydiae, cervicitis, and abnormal Papanicolaou smears. *Obstet Gynecol* 1979; **53**: 27-30.
8. Paavonen J, Saikku P, Vesterinen E, Meyer B, Vartiainen E, Saksela E. Genital chlamydial infections in patients attending a gynaecological outpatient clinic. *Br J Vener Dis* 1978; **54**: 257-61.
9. Costa S, Guerra B, Diana R, Antonini MP, Cevenini R, Donati M, Rumpianesi F, Landini MP. Sui rapporti tra patologia cervicale e *Chlamydia trachomatis*. *Riv Ital Ginecol* 1980; **58**: 415-25.
10. Cevenini R, Negosanti M, La Placa M. First data on the frequency of *Chlamydia trachomatis* in non-gonococcal urethritis in Italy. *Microbiologica* 1978; **1**: 107-10.
11. Wentworth BB, Alexander ER. Isolation of *Chlamydia trachomatis* by use of 5-iodo-2'-deoxyuridine-treated cells. *Appl Microbiol* 1974; **27**: 912-4.
12. Richmond SJ, Caul EO. Fluorescent antibody studies in chlamydial infections. *J Clin Microbiol* 1975; **1**: 345-52.
13. Cevenini R, Donati M, Landini MP, Rumpianesi F, Brignola C, Benatti A, Campieri M, Lanfranchi GA. Antibodies against *Chlamydia trachomatis* in patients with Crohn's disease. *Microbiologica* 1980; **3**: 109-13.
14. Wurch T, Isaac JP. Nouvelle technique de coloration histologique differentielle en trois temps pour le diagnostic des cancers des voies temps-genitales de la femme par la methode cytologique. *Rev Fr Gynecol Obstet* 1951; **319**: 9-10.
15. Papanicolaou GN, Traut HF. Diagnostic value of vaginal smears in carcinoma of the uterus. *Am J Obstet Gynecol* 1941; **42**: 193-5.
16. Carrera JM, Dexeu S, Coupez F. *Tratado y Atlas de Colposcopia*. Barcelona: Salvat Editores: 1974.